510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

k061588

B. Purpose for Submission:

New device

C. Measurand:

Homocysteine

D. Type of Test:

Quantitative, homogeneous enzymatic assay

E. Applicant:

Ortho-Clinical Diagnostics, Inc.

F. Proprietary and Established Names:

VITROS Chemistry Products HCY Reagent, VITROS Chemistry Products Calibrator Kit 27, VITROS HCY Performance Verifiers I, II, and III

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
Urinary Homocysteine (Nonquantitative) Test System (LPS)	Class II	21 CFR 862.1377, Urinary homocysteine (nonquantitative) test system.	75 Clinical Chemistry (CH)
Product Code	**Classification**	**Regulation Section**	**Panel**
Calibrators, Secondary (JIT)	Class II	21 CFR 862.1150, Calibrator	75 Clinical Chemistry (CH)
Product Code	**Classification**	**Regulation Section**	**Panel**
Single (Specified) analyte controls (assayed and unassayed) (JJX)	Class I	21 CFR 862.1660, Quality Control Material (assayed and unassayed)	75 Clinical Chemistry (CH)

H. Intended Use:

1. Intended use(s):

   VITROS Chemistry Products HCY Reagent: For *in vitro* diagnostic use only. VITROS Chemistry Products HCY Reagent is used to quantitatively measure total homocysteine (HCY) concentration in human serum and plasma. Serum and plasma homocysteine levels can assist in the diagnosis and treatment of patients suspected of having hyperhomocysteinemia and homocystinuria.

   VITROS Chemistry Products Calibrator Kit 27: For *in vitro* diagnostic use only. VITROS Chemistry Products Calibrator Kit 27 is used to calibrate VITROS 5,1 FS Chemistry Systems for the quantitative measurement of homocysteine (HCY).

   VITROS Chemistry Products HCY Performance Verifiers I, II & III: For *in vitro* diagnostic use only. VITROS Chemistry Products HCY Performance Verifiers are assayed controls used to monitor performance of VITROS HCY Reagents on VITROS 5,1 FS Chemistry Systems.

2. Indication(s) for use:
   See Intended Use above.

3. Special conditions for use statement(s):
   Prescription use only

4. Special instrument requirements:

   The device is intended for use with the VITROS 5,1 FS Chemistry System. The VITROS 5,1 FS Chemistry System was cleared for market use in k031924.

I. Device Description:

The VITROS Chemistry Products HCY Reagent consists of two dual chambered reagent packs containing three ready-to-use liquid reagents. VITROS Chemistry Products FS Diluent Pack 2 (Saline/BSA) is a common reagent that is used by multiple assays on the VITROS 5,1 FS Chemistry System. This is a dual chambered package containing two ready-to-use liquid diluents. Diluent 1 is prepared from processed water to which inorganic salt has been added. Diluent 2 is prepared from processed water to which bovine serum albumin, inorganic salts and preservatives have been added. Diluent 1 contains saline (154 mM NaCl), which is used to dilute elevated homocysteine (HCY) samples on the analyzer.

VITROS Chemistry Products Calibrator Kit 27 is a two level fluid prepared from an aqueous solution containing amino acids and inorganic acid. These standards are used

to calibrate VITROS 5,1 FS Chemistry Systems for the quantitative measurement of homocysteine (HCY).

VITROS HCY Performance Verifiers I, II and III are prepared from processed human serum to which amino acid and preservative have been added. These are assayed controls used to monitor performance of VITROS HCY Reagent on VITROS 5,1 FS Chemistry Systems.

J. Substantial Equivalence Information:

Predicate	**k993107 Bio-Rad Homocysteine by HPLC Test**

Describe the item being compared

The Bio-Rad Homocysteine by HPLC test is based on precolumn derivatization and a 5 minute chromatography. The Bio-Rad Homocysteine by HPLC test is intended for the quantitative determination of total homocysteine in human plasma or serum.

Device Similarities

Device Characteristic	**VITROS HCY (New device)** **BIO-RAD HOMOCYSTEINE by HPLC test (Predicate device)**
Intended use	For *in-vitro* diagnostic use. Quantitative determination of total homocysteine in human serum or plasma
Analyte measured	Homocysteine
Sample type	Serum or Plasma
Measurement	Quantitative

Device Differences

Device Characteristics	**VITROS HCY (New device)**	**BIO-RAD HOMOCYSTEINE by HPLC test (Predicate device)**
Reportable range	1.0 – 50.0 µmol/L	0.5 – 100 µmol/L
Sensitivity	1.0 µmol/L	0.5 µmol/L
Calibrator levels	Two levels (0, 27 µmol/L)	Single level (15-20 µmol/L)
Calibrator format	Liquid	Lyophilized
Calibrator matrix	Aqueous solution containing amino acids and inorganic acid	Human Serum
Instrumentation	Automated clinical chemistry analyzer	Isocratic HPLC System

Reference Interval	Males: 6.6 – 14.8 µmol/L Females: 4.7 – 12.6 µmol/L	< 15 µmol/L
Method	Homogeneous Enzymatic	Chromatography

Predicate	**k052819 VITROS Chemistry Products AAT Performance Verifiers I, II, & III**
Describe the item being compared	
The VITROS Chemistry Products AAT Performance Verifiers I, II and III are prepared from processed human serum to which inorganic salts, buffers, and preservatives have been added. These are assayed controls used to monitor performance of VITROS AAT Reagent on VITROS 5,1 FS Chemistry Systems.	
Device Similarities	
Indications for use	Assayed controls used to monitor assay performance on VITROS 5,1 FS Chemistry Systems
Matrix	Prepared from processed human serum with preservatives added.
Product type	Assayed control
Format	Liquid
Number of levels	Three
Device Differences	
Device Characteristic	**VITROS HCY Performance Verifiers (new device)** / **VITROS AAT Performance Verifiers (predicate device)**
Analyte measure	Homocysteine / alpha 1-Antitrypsin (AAT)

K. Standard/Guidance Document Referenced (if applicable):

STANDARDS
Title and Reference Number
Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline (EP5-A)
Interference Testing in Clinical Chemistry; Approved Guideline (EP 7-A)
Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline (EP09-A2)
How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition (C28-A2)
Other Standards

None			
GUIDANCE			
Document Title	**Office**	**Division**	
None			

L. Test Principle:

The quantitative measurement of homocysteine (HCY) is performed using the VITROS Chemistry Products HCY Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 27 and on the VITROS 5,1 FS Chemistry Systems. The VITROS Chemistry Products HCY Reagent consists of two dual chambered reagent packs containing three ready-to-use liquid reagents. Disulfide linked homocysteine (oxidized forms) in the sample is reduced by Tris (2-Carboxyethyl) phosphine hydrochloride (TCEP) to form reduced homocysteine. Reduced homocysteine reacts with serine in the presence of cystathionine b-synthase (CBS) to form L-cystathionine. L-cystathionine is broken down by cystathionine b-lyase (CBL) to produce homocysteine, pyruvate and ammonia. Pyruvate is reduced to lactate by lactate dehydrogenase (LDH) using NADH as coenzyme. The concentration of homocysteine is directly proportional to the amount of NADH converted to NAD^+ and is measured spectrophotometrically at 340 nm. Once a calibration has been performed, the homocysteine concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbance obtained in the assay of the sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

      Precision was evaluated with quality control materials and a human serum pool on the VITROS 5,1 FS Chemistry System following NCCLS Protocol EP5.

VITROS Chemistry Products HCY Reagent Precision (Serum)

System	SI Units (µmol/L)					
	Mean Conc. (µmol/L)	Within-Day SD*	Within-Lab SD**	Within-Lab %CV	No. Days	No. Obser
VITROS 5,1 FS	7.0	0.21	0.24	3.4	20	80
	11.9	0.23	0.27	2.3	20	80
	41.8	0.33	0.44	1.1	20	80
	23.7	0.24	0.30	1.3	20	80
	48.0	0.52	0.66	1.4	20	80

*Within Day precision was determined using two-runs/ day with two replicates per run

** Within Lab precision was determined using a single lot of reagents on a single analyzer, calibrating once a week (4 calibrations).

b. *Linearity/assay reportable range:*

The evaluation of the linearity of the VITROS HCY assay was performed based on NCCLS EP6-A. Three lots of VITROS Chemistry Products HCY Reagents were tested using a series of admixtures with concentrations of 0.50 µmol/L to 71.0 µmol/L. The VITROS Chemistry Products HCY Reagent linear range was determined to be 0.50 µmol/L to 71.0 µmol/L and the reportable range was chosen by the applicant to be 1.0 µmol/L to 50.0 µmol/L.

An on-board dilution study of patient samples with saline was performed using 10 samples with a dilution factor of 2 and 10 samples with a dilution factor of 3. The recovery ranged from 96.9% to 100.0% using the dilution factor of 2 and ranged from 92.4% to 98.5% using the dilution factor of 3.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Traceability

Both the Calibrators and Verifiers are value assigned by performing multiple determinations using the VITROS 5,1 FS Chemistry Systems and the VITROS Chemistry Products HCY Reagent. The VITROS Chemistry Products Calibrator Kit 27 is traceable to NIST SRM 1955.

Stability

The long term (shelf life) stability of the VITROS HCY reagents, calibrators and verifiers were tested on a VITROS 5,1 FS Chemistry System using a real time study extending at least one month beyond the selected expiration date. Testing was done at intervals of 0, 1, 2, 3 months and is still ongoing. The

VITROS HCY reagents, calibrators and verifiers are stored at refrigerated (2-8^{o}C).

The opened vial (in use) stability of the VITROS HCY reagents, calibrators and verifiers were tested on a VITROS 5,1 FS Chemistry System. Testing was done at intervals of 0, 7, 14 days and is still ongoing.

d. Detection limit:

The LOB specimen tested was a homocysteine-free serum pool obtained from Golden West Biologics Inc, Temecula, CA. The LOD specimen tested was consisted of two homocysteine serum pools spiked with homocysteine to nominal concentrations of 0.01 and 0.48 µmol/L. The Limit of Blank (LOB), Limit of Detection (LOD), and Limit of Quantitation (LOQ) were determined following NCCLS EP17-A. The lower linear range of the VITROS HCY assay was determined to be 0.5 µmol/L based on the results of the linearity evaluation. The value that will be the claimed analytical limit (reportable range) at low levels is 1.0 µmol/L based on linearity, LOB, LOD, and LOQ evaluations.

e. Analytical specificity:

The specificity of the VITROS HCY reagent was evaluated by testing the following substances at homocysteine concentrations of approximately 15.0 to 38.0 µmol/L using protocols based on NCCLS Protocol EP7-A and found not to interfere, bias <13.6%, at the concentrations shown below.

Compound	**Concentration**	
Acetaminophen	20 mg/dL	Acetaminophen
N-Acetyl L-Cysteine	326 µg/L	N-Acetyl L-Cysteine
Adenosine	6.7 g/dL	Adenosine
Amoxicillin	20 µg/mL	Amoxicillin
Ampicillin	100 mg/dL	Ampicillin
Ascorbic Acid (L)	10 mg/dL	Ascorbic Acid (L)
Aspirin	50 mg/dL	Aspirin
Atorvastatin	300 ng/mL	Atorvastatin
Bilirubin	30 mg/dL	Bilirubin
Caffeine	10 mg/dL	Caffeine
Clopidogrel hydrogensulfate	50 µg/mL	Clopidogrel hydrogensulfate
Creatinine	30 mg/dL	Creatinine
L-Cysteine	2.4 g/dL	L-Cysteine

Compound	Concentration	
Doxycyclin	5 mg/dL	Doxycyclin
D-penicillamine	8 µg/mL	D-penicillamine
Enalapril Maleate	120 ng/mL	Enalapril Maleate
Gemfibrozil	50 µg/mL	Gemfibrozil
Gentamycin Sulfate	120 µg/mL	Gentamycin Sulfate
Glutathione	0.6 g/L	Glutathione
DL-Homocysteine thiolactone	0.77 mg/dL	DL-Homocysteine thiolactone
Hemoglobin	500 mg/dL	Hemoglobin
Hydrochlorothiazide	2 µg/mL	Hydrochlorothiazide
Ibuprofen	40 mg/dL	Ibuprofen
Intralipid	500 mg/dL	Intralipid
L-Methionine	29.8 mg/dL	L-Methionine
S-(5.-Adenosyl)-L-Methionine p-toluene sulfonate	79.9 mg/dL	S-(5.-Adenosyl)-L-Methionine p-toluene sulfonate
Metronidazole	20 mg/dL	Metronidazole
Nicotine	2 mg/dL	Nicotine
Nifedipine	200 ng/mL	Nifedipine
Procainamide	100 µg/mL	Procainamide
Propanolol	5 µg/mL	Propanolol
Protein (Serum)	9.3 g/dL	Protein (Serum)
Pyruvic Acid	17.6 mg/dL	Pyruvic Acid
Rheumatoid Factor	373 IU/mL	Rheumatoid Factor
Theophylline	250 µg/mL	Theophylline
Triglycerides	908 mg/dL	Triglycerides
Urea	500 mg/dL	Urea
Warfarin	10 µg/mL	Warfarin

Known Interferences

• Patient samples containing cystathionine (reference interval: 0.065 to 0.301 µmol/L) show positive equimolar interference with the VITROS Chemistry

Products HCY assay. For example, a sample containing 10.0 µmol/L homocysteine and 0.3µmol/L cystathionine will generate a VITROS HCY result of 10.3 µmol/L. Elevated cystathionine levels can be observed in patients with certain conditions, such as renal disease, folate deficiency, and cystathionine beta-lyase deficiency.

• For samples that generate a Sample Integrity T-index flag, refer to the Sample Dilution section in the package insert.

Other Limitations

Results should be interpreted with caution. Certain drugs and clinical conditions are known to alter homocysteine concentration *in vivo*. For example, patients who are taking methotrexate, carbamazepine, phenytoin, nitrous oxide, anticonvulsants, or 6-azuridine triacetate, may have higher levels of homocysteine due to metabolic interference with homocysteine metabolism. For additional information, refer to one of the published summaries cited in the package insert.

f.. Assay cut-off:

None

2. Comparison studies:

a. Method comparison with predicate device:

A method comparison study was performed on 93 human serum samples on the VITROS 5,1 FS Chemistry Systems and a commercially available method, based on NCCLS Protocol EP9. Three reagent lots were used for the testing and all samples were analyzed in triplicate. A least squares linear regression analysis demonstrated the following relationship: y=0.98 x + 1.0 µmol/L, with a correlation coefficient = 0.97 where y= results obtained using the VITROS HCY assay and x= results obtained with the commercially available system BIO-RAD homocysteine by HPLC assay.

b. Matrix comparison:

Matrix comparison studies were performed by collecting fresh whole blood from a total of 95 donors. Samples were collected into plain glass, lithium-heparin plasma, K2EDTA plasma, K3EDTA plasma, and serum separator tubes at variable fill volumes and pairings and then centrifuged and the serum separated within 1 hr of collection. For each test condition (specimen type or stability), assay value difference were calculated from the baseline (serum in plain glass for specimen collection and fresh sample for stability). The mean and range of the % differences, across all samples, for each condition were calculated. For each storage condition, means were calculated and the bias from fresh results was compared to the applicant's acceptance criteria for

determination of acceptability. All the specimen types tested were acceptable at the specimen matrix and stability claims except the plasma samples must be collected in plasma tubes that are at least half full. The information that smaller sample volumes can result in negative bias is presented in the package insert.

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   None

4. Clinical cut-off:

   None

5. Expected values/Reference range:

   Reference intervals were determined for the VITROS HCY assay following NCCLS Guideline C28-A2. The reference interval is based on the central 95 percent of homocysteine values from an Ortho Clinical Diagnostics, Inc. study of 106 healthy female and 99 healthy male adults. Testing was performed on three VITROS 5,1 FS Chemistry System analyzers using 3 lots of VITROS HCY reagents. The reference range for males is 6.6-14.8 µmol/L and for females is 4.7-12.6 µmol/L.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.